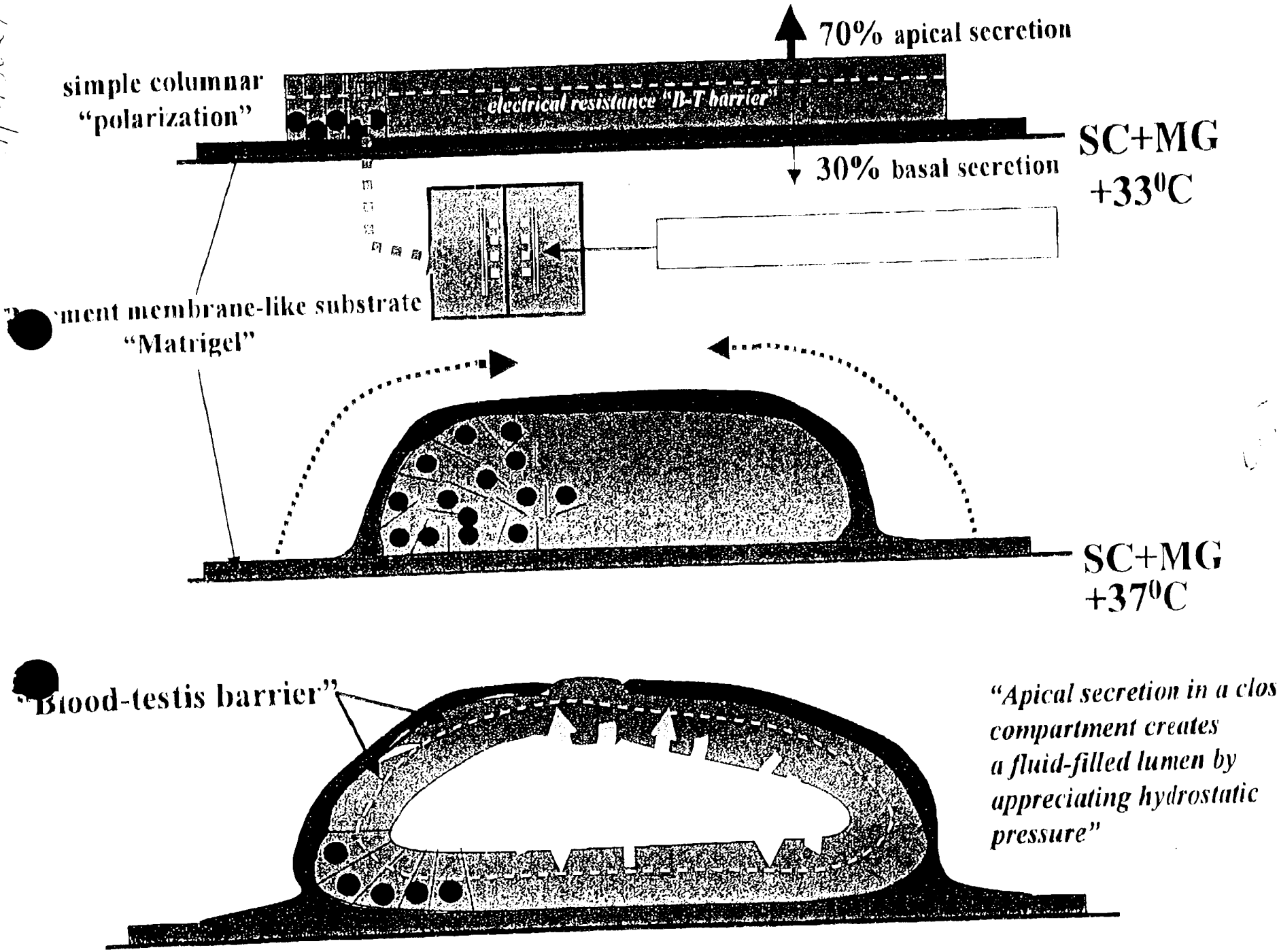
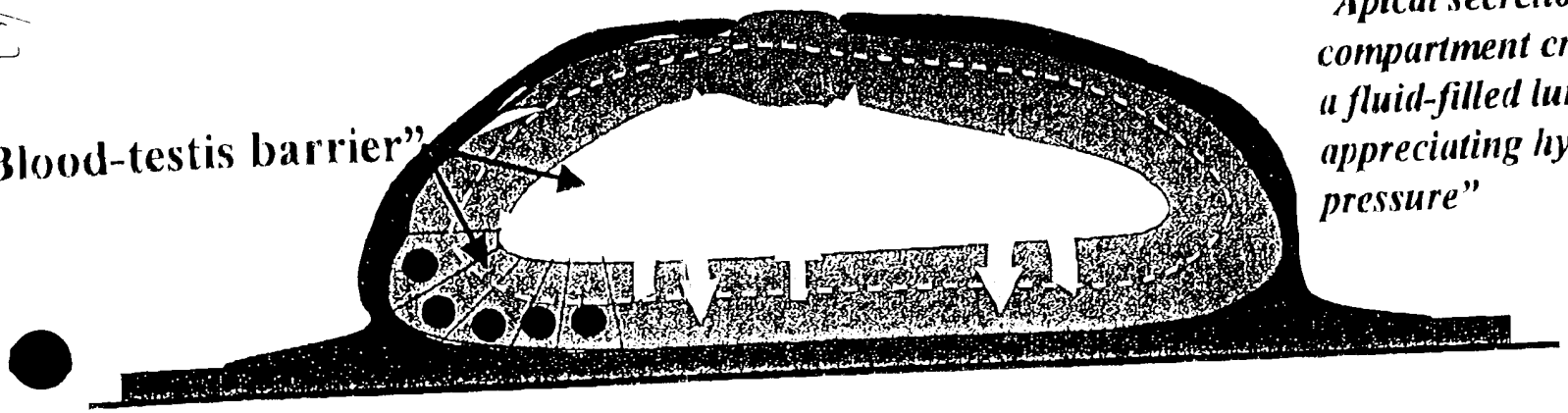


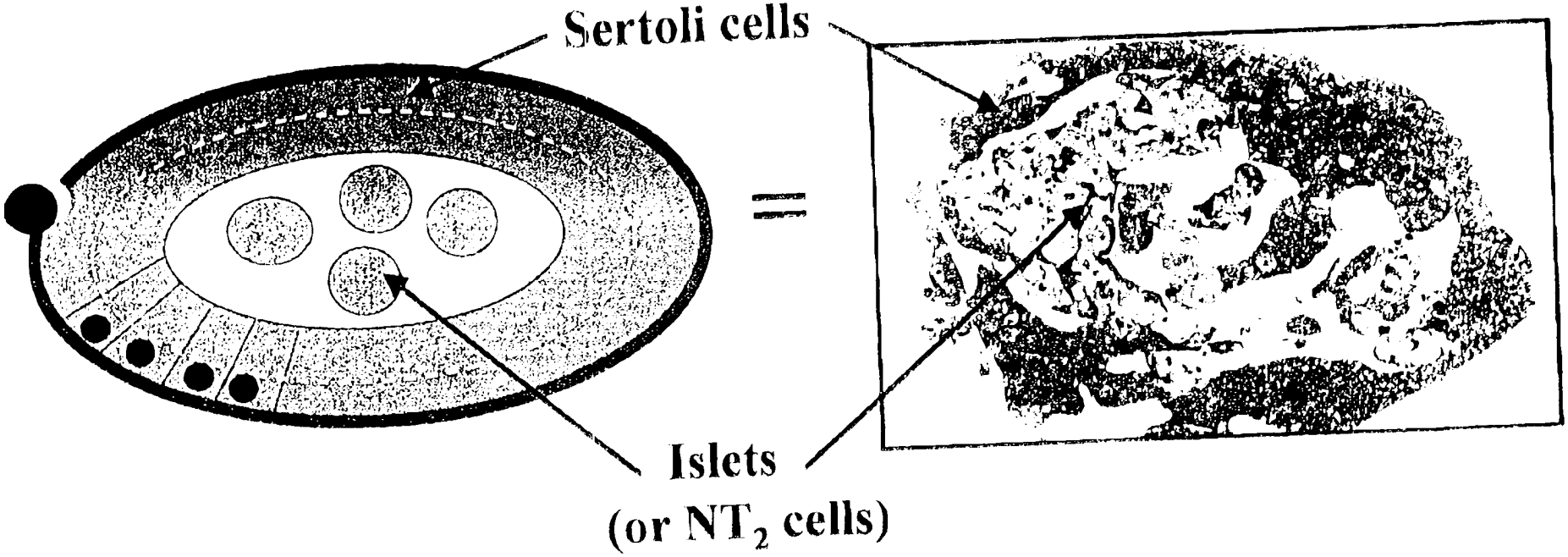
Conventional Culture

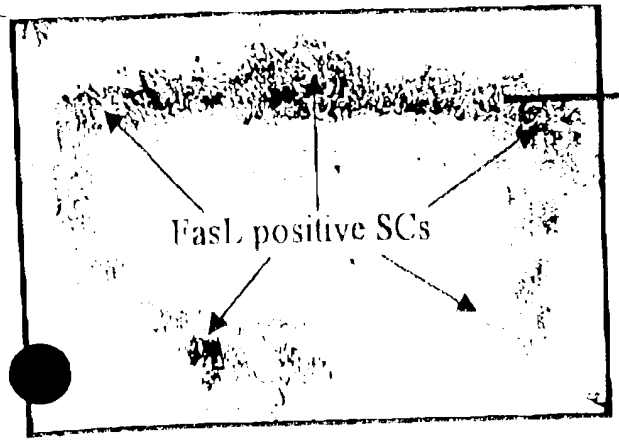


"Apical secretion in a closed compartment creates a fluid-filled lumen by appreciating hydrostatic pressure"

Microgravity Coculture

"Microgravity coculture results in th integration of therapeutic cells into Sertoli cell biochambers "



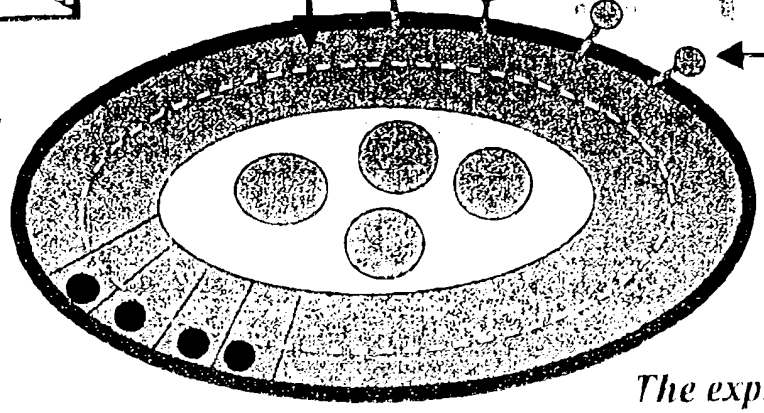


Activated T-lymphocytes with upregulated Fas receptors

FasL-Fas induced apoptosis of lymphocytes

FasL

Positive FasL immonstaining identifies Sertoli cells and suggests a mechanism by which they may effect immune suppression at the graft site.



Sertoli Biochamber

The expression of FasL by Sertoli cells induces apoptosis of the invading immune cells by binding to the upregulated Fas receptors on these activated T-lymphoctys. This results in the attrition of these immune cells at the graft site thereby downregulating the immue respones-this by an already well-defined mechanism occuring naturally in the mammalian system.

Addition to Disclosure "Sertoli Biochambers"
Cameron, Don F. et al.

Isolated Sertoli cells from peripubertal rats and pancreatic islets from neonatal pigs were cocultured by conventional culture technology in the same medium described for the HARV simulated microgravity coculture. Sertoli cells were pre-plated 48 hours on plastic or Matrigel substrates. Pre-treated isolated pig islets were added to the Sertoli cell-enriched monoculture 24 hours later. This Sertoli-Islet coculture was incubated at 37°C and by 24 hr islets attached to and integrated into the underlying Sertoli cells. Within antother 48-72hrs. Sertoli cells reorganized into spherical or chord-like aggregates. This process was enhanced for those cocultures in which Sertoli cells had been plated on the Matrigel. Islets appeared to retain their structural integrity better in the non-Matrigel cocultures (Fig 1) than in the cocultures not having a Matrigel substrate (Fig 2). Tissue constructs of Sertoli cells and pancreatic islet cells can be created in conventional coculture in a similar manner as that observed in simulated microgravity coculture.

Fig 1 Sertoli cell (SCs) and islets (arrows) in a Sertoli-islet tissue construct created in conventional coculture. B-cells are immunostained for insulin.

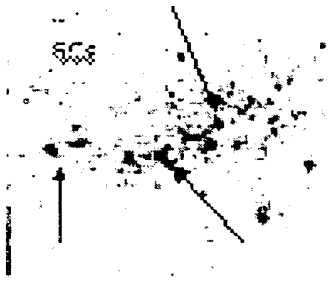
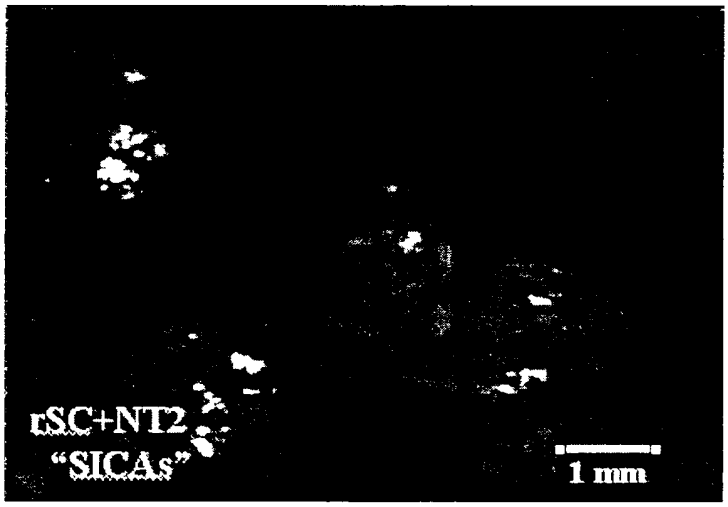


Fig 2. Sertoli cells (SCs) and B-cells (arrows) in a Sertoli-islet tissue construct created in conventional coculture. B-cells are immunostained for insulin.

1. Sanberg, P.R., C.V. Borlongan, A.I. Othberg, S. Saporta, T.B. Freeman and D.F. Cameron. Testis-derived Sertoli cells have a trophic effect on dopamine neurons and alleviate hemiparkinsonism in rats. Nature medicine, 3(10 :1129-1132.

Figure 1.

1 Week HARV Coculture - rSCs + NT2



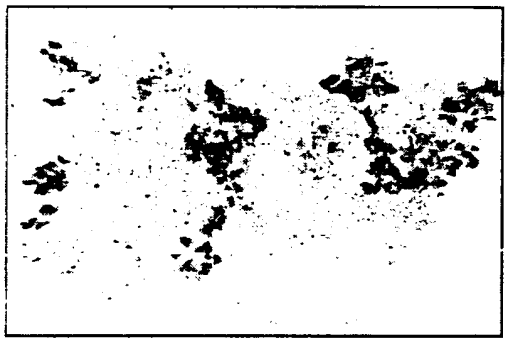
Sertoli-Neuron-Aggregatee-Cells (SNACs) form *in vitro* following coculture of rat Sertoli cells and NT2 neuros in simulated microgravity utilizing the High Aspect Rotation Velocity (HARV) bioreactor.

Figure 2.

1 Week HARV Coculture - rSCs + hNT2 (neurons)

NT2 + SC
FasL

NT2 + SC
hNuMu



Immucytochemical staining of mouse FasL and human nuclear matrix proteins in rSertoli-hNeuron Aggregated Cells (SNACs) following HARV incubated cocultures.